

# SIGNIFICANTLY INCREASED RECOVERY OF INTESTINAL PARASITES ON ROUTINE STOOL SPECIMEN EVALUATION

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**Abstract.** Three hundred thirty-six stool samples from October 2001 through October 2002 were analyzed for the presence of intestinal parasites. Fifty-six of these (16.7%) were positive for a total of 66 parasites; 65/66 (98.5%) were detected by iodine and dimethyl sulfoxide-modified acid-fast (DMSO-mAFB) stained smears of fresh and formalin-ethylacetate sedimentation concentrated samples. Saline, iodine, and DMSO-mAFB stained smears of fresh stool samples alone detected significantly fewer parasites, finding only 50/66 (75.8%) ( $p < 0.05$ ). Stool samples analyzed by trichrome stained specimens preserved in Zinc sulfate polyvinyl alcohol (Zinc PVA) detected only 41/66 (62.2%) of the parasites. In our study population, it was necessary to perform the National Committee for Clinical Laboratory Standard (NCCLS) recommended to accurately detect intestinal parasites. The concentration technique is simple and significantly increased the detection of intestinal parasites.

## INTRODUCTION

The examination of stool specimens for the detection of intestinal parasites in our laboratory involves six microscopic analyses: direct saline and iodine wet mounts of fresh stool, a DMSO-mAFB stained smear of fresh stool, an iodine wet mount and DMSO-mAFB stained smear of a sample concentrated by the formalin-ethylacetate sedimentation concentration technique from a 10% buffered formalin preserved stool, and a trichrome stained smear from a Zinc sulfate polyvinyl alcohol (Zinc PVA) preserved stool (Bronsdon *et al*, 1984; Garcia *et al*, 2001; James and Carol, 1999). Many laboratories, however, are now routinely performing only direct saline and iodine wet mounts of fresh stool for the detection of intestinal parasites. Material costs, labor costs, inconvenience, complexity, and the toxicity of formaldehyde are factors in this decision. While each laboratory must decide

for itself which tests it will run, this decision must be based not only upon which procedures are requested by the physicians who use the laboratory services, the lag time during specimen collection and submission to laboratory, and the prevalence of different pathogens in their population, but must also take into account the accuracy of the diagnostic protocol (Hale *et al*, 1996; Garcia *et al*, 2001).

This study was performed to evaluate the necessity of performing all of the NCCLS recommended procedures (especially the concentration technique) that are described above. To accomplish this, the parasites detected by the concentration technique of specimens preserved in 10% buffered formalin was retrospectively compared with those of the saline and iodine direct wet mounts, and DMSO-mAFB stained smears prepared from fresh stools.

## MATERIALS AND METHODS

After informed consents were obtained, stool specimens were collected from children 3 months to 5 years of age from the Sangkhla Buri district in Kanchanaburi, a western province of Thailand along the Thai-Myanmar border. All stool specimens were processed by mixing one portion of stool to three portions of preservative

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Table 1  
 Detection of ova and parasites from fresh stool, iodine stained concentrated stool, and via trichrome staining.

Parasite (n)	Parasites detected		
	Direct smear/ DMSO-mAFB fresh stool (%)	Iodine-stained/DMSO-mAFB concentrated stool (%)	Trichrome staining (%)
<i>A. lumbricoides</i> (13)	8 (61.5)	12 (92.3)	0
<i>B. hominis</i> (1)	0	1 (100)	1 (100)
<i>Cryptosporidium</i> spp (5)	4 (80)	5 (100)	0
<i>Cyclospora</i> spp (6)	6 (100)	6 (100)	0
<i>G. lamblia</i> (40)	32 (80)	40 (100)	40 (100)
Total (66)	50 (75.8)	65 (98.5)	41 (62.1)

into 10% buffered formalin and Zinc PVA filled vials (James and Carol, 1999). Saline and an iodine wet mounts of fresh stools were also prepared. These slides of fresh stool smears were fixed in absolute methanol for 10 minutes and air dried before weekly shipment to AFRIMS. DMSO-mAFB staining (Bronsdon *et al*, 1984) for detecting *Cryptosporidium* and *Cyclospora* oocysts together with an iodine wet mount were prepared from the formalin preserved specimens after performing a formalin-ethylacetate sedimentation concentration. Trichrome staining was performed on the stool specimens preserved in Zinc PVA (Bronsdon *et al*, 1984; James and Carol, 1999; Garcia *et al*, 2001).

The chi-square test for independent samples was used for statistical analysis of the results (Dean *et al*, 1990). A p-value of <0.05 was selected as the minimum level denoting significance.

## RESULTS

From 16 October 2001 through 31 October 2002, 336 fresh and preserved specimens from 336 patients were analyzed for ova and parasites. A total of 66 parasites were detected in 56 (16.7%) of the specimens. Of the 66 parasites found, the concentration technique permitted the detection of 98.5% (65 of 66) of the total parasites found, while direct smears and trichrome stained smears allowed the detection of only 75.8% (50 of 66) and 62.1% (41 of 66) of the total parasites, respectively.

## DISCUSSION

In this study, 98.5% (65 of 66) of the total parasites were found by a simple iodine wet smear and modified acid-fast stained smear of concentrated stool specimens. Significantly fewer (75.8%) of these parasites were correctly identified if the examinations were performed only on fresh stool samples. Additionally, we found that the addition of trichrome staining did not identify any additional pathogenic parasites. Trichrome staining alone correctly identified 41/41(100%) of pathogenic protozoa, excluding *Cryptosporidium* spp and *Cyclospora* spp. These species stained poorly with trichrome. *Ascaris* eggs stained too dark and were distorted by the staining, so that they could not be identified. We did find the trichrome staining useful for confirmation of *G. lamblia* cysts by facilitating the identification of their internal structures. We also noted that nonpathogenic ameba (such as *E.coli* cysts, *E.nana* cysts, and *I.buttschlii* cysts) could be more easily found on trichrome stained smears than in iodine wet smears of a concentrated specimen.

The results from this study differ from some other recent studies looking at the same techniques. In 2,204 specimens positive for pathogenic protozoa, a detection rate of only 29.2% was found using a wet mount of formalin-ether concentrated stool, compared to 99.8% with trichrome staining. (Garcia *et al*, 1979). A detection rate of only 47.0% for iodine wet mounts of concentrated stool as compared to 99.2% for

trichrome staining was reported. They went so far as to suggest the use of a single trichrome stained slide from a concentrated stool specimen as the sole means for routine detection of intestinal parasites (James and Carol, 1999). Similarly, the suggestion of one trichrome stained smear of a concentrated stool specimen from a sample from only a single specimen of PVA preserved stool increased the detection of parasites and offered a cost savings in both material and labor (Hale *et al*, 1996). However, it is reported that only 14.6% of helminth ova and larvae can be detected by trichrome staining alone in concentrated specimens (Wood *et al*, 1982). Some parasites, including hookworm eggs, *Trichuris* eggs and *G.lambli*a, may not concentrate as well from stool specimens preserved in PVA as compared to those preserved in formalin (Garcia *et al*, 2001). While trichrome staining produces uniformly well stained smears of intestinal protozoa, human cells, yeast cells, and artifact material, and is the primary method for the confirmation of intestinal trophozoite and cyst forms of protozoa, it is not recommended for staining helminth eggs or larvae (Garcia *et al*, 2001; NCCLS, 1997). They are often too dark or too distorted to be successfully identified. With some highly experienced laboratory personnel, however, they are occasionally recognized and identified by this method. It is therefore recommended that an iodine wet smear preparation, and a modified acid-fast stained smear from the concentrated stool specimen be used for the identification of helminth eggs and larvae and for *Cryptosporidium* and *Cyclospora* oocysts, which will generally not be recognized on a trichrome stained smear.

This study, adds to the call for each laboratory to decide for itself which diagnostic methods will best serve their populations. The more methods performed, the more parasites will be detected, but there will be a corresponding increase in cost and complexity. For our population in Thailand with its large population of immigrants, poor sanitation, and AIDS patients, a single trichrome stained smear of a stool specimens has proven not to be adequate. This retrospective analysis indicates that in our study population, the routine use of the NCCLS rec-

ommended four procedures was necessary and appropriate to successfully detect and confirm all intestinal parasites, including protozoa, helminth eggs, *Cryptosporidium* and *Cyclospora* oocysts.

## ACKNOWLEDGEMENTS

We would like to express our appreciation to the Director of Kwai River Christian Hospital, the staff in the hospital, all the staff in the Enteric Diseases Department, and to Caroline Dorworth Fukuda for her proofreading of this manuscript.

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